The Examiner has objected to the title of the invention as being non-descriptive. Applicants have amended the specification to include a title which more precisely describes the claimed invention in accordance with the Examiner's suggestions, and is not intended in any way to modify the scope of the claimed invention.

The Examiner has objected to the Abstract as being non-descriptive. Applicants have, therefore, amended the Abstract by identifying the modifications of the oligonucleotides and their use.

The Examiner has objected to the specification and rejected claims 13-24 under 35 U.S.C. § 112, first paragraph. Applicants respectfully traverse this objection and rejection.

Antisense methodology is the complementary hybridization of relatively short oligonucleotides to single-stranded mRNA or single stranded DNA such that the normal, essential functions of these intracellular nucleic acids are disrupted. Page 2, lines 3-7. The present invention is a method of use of an improved antisense oligonucleotide. While the antisense oligonucleotide has been modified, the "antisense mechanism", i.e., hybridization leading to disruption of the function of intracellular nucleic acids, remains the same. Applicants have shown that antisense oligonucleotides of the

present invention hybridize with fidelity to target sequences (Page 35, lines 1-35; Page 36, lines 1-18).

In an earnest effort to advance the prosecution of this case, Applicants have amended the claims to specify that an effective amount of the composition is administered. Those skilled in the art are well versed in methods for administration of antisense oligonucleotides to effect inhibition of protein expression in a variety of species, and it is well known that the effective amount will vary by species and mode of administration. However, it is within the skill of a routineer to determine such effective amounts. For example, Markus-Sekura et al. (page 5754) teach a mode of administration of antisense oligonucleotides wherein CV-1 cells were incubated with 30μM oligo-methylphosphonate to inhibit expression of chloramphenicol. Other modes of administration are described by U.S. Patent No. 4,689,320 (Kaji et al.) which disclose injectionable preparations and suppositories containing 1-10mg of oligonucleotide per ampoule or capsule. For human patients, the suggested effective amount is from about 10-20 μg/kg to 1000-2000 μg/kg body weight, depending on common variables such as age, weight, general state of health, etc. Kaji et al. further describe treatment of mice infected with HSV-1(F strain). Mice were injected with 200μl solutions of 1% penicillin and streptomycin, 2mM glutamic acid, and from .224 to 2.24 μg oligonucleotides intraperitoneally

once/day for 6 days. In addition, corneas of mice infected with HSV-1 (F strain) were treated by dropping 10μl of MEM containing 22.4μg of oligonucleotide into infected eyes every other day. Miller and Ts'o, *Anti-Cancer Drug Design*, 2:117-128 (1987), disclose applying oligonucleoside methylphosphonate in a cream to an HSV-1 infected ear of a mouse to reduce the yield of virus in both the skin region and the ganglion region of the ear. A review article by Zon et al. *Pharmaceutical Research*, 5(9):539-549 (1988) describes oligonucleotide-mediated protein inhibition by a number of groups. Inhibition of protein translation in virus infected mouse L cells was achieved by the administration of 100-150 μM oligonucleoside methylphosphonates, and 50%, 90% and 99% decreases of HSV-1 virus titre in African green monkey cells was achieved by the administration of 25μM, 75μM, and 300μM oligonucleoside methylphosphonates, respectively. Inhibition of Rous sarcoma virus in chick embryo fibroblast tissue culture occurred at the low micromolecular range of 10-mer oligodeoxyribonucleotide concentration. In addition, inhibition of HIV was observed in cultured human cells at 9μM 20-mer oligodeoxyribonucleotide by one investigator, and at 1μM 28-mer phosphorothioate oligonucleotide by a second investigator. Applicants respectfully submit that one skilled in the art would be familiar with a variety of effective treatments and would further be familiar with the need for variations in such

treatments to accommodate different species and subjects. Therefore, Applicants respectfully request withdrawal of the § 112 rejection.

A copy of the reference by Miller and Ts'O et al. has previously been provided to Examiner in the Information Disclosure Statement submitted December 19, 1990. Copies of Kaji et al. and Zon at al. are being submitted with Supplemental IDS submitted herewith.

The Examiner has rejected claims 13-24 under § 112, first and second paragraphs. Applicants respectfully traverse this rejection. Applicants have directed their invention to organisms having a selected sequence of RNA or DNA coding for a selected protein. It is Applicants' intention to encompass *all* organisms where RNA or DNA code for the selected protein, such as, for example, bacteria, viruses, and animals in which antisense oligonucleotides have been shown to modulate gene expression. For example, at page 9, lines 12-26, Applicants describe the treatment of animals suffering from a disease effected by a selected protein. Since antisense oligonucleotides have been found to be effective in a broad range of cells, Applicants respectfully submit that they have sufficiently pointed out and distinctly claimed the subject matter which Applicants regard as their invention and, therefore, respectfully request withdrawal of this § 112 rejection.

The Examiner has rejected claims 13-24 under § 103 as being unpatentable over Ikehara et al. and Marcus-Sekura et al. Applicants respectfully traverse this rejection.

Marcus-Sekura et al. compared several classes of oligonucleotides for their ability to inhibit synthesis of chloramphenicol acetyl transferase (CAT). They recognized that normal oligonucleotides have relatively short half-lives due to nuclease degradation. Marcus-Sekura et al. suggested that replacement or modification of the oligonucleotide sugar-phosphate backbone by or to a phosphorothioate or methylphosphonate can increase the oligonucleotide biological lifetime, enhance permeability into cells, and strengthen binding to polynucleotide targets under physiological conditions.

Ikehara et al. synthesized poly (2'-chloro-2'-deoxyadenylic acid) and poly (2'-bromo-2'-deoxyadenylic acid) and found that the 2'-substituents exert significant effect on thermal stability of the polynucleotides. It was found that 2'-substituents had a destabilizing affect on the thermal stability of the molecule by itself and when complexed with other polynucleotide strands. The polynucleotides were also determined to be resistant wholly to ribonuclease M^{11} and partly to snake venom phosphodiesterase.

Claims of the present invention are directed to a method for modulating the production of a protein by an organism

by contacting the organism with an oligonucleotide or oligonucleotide analog having a sequence of nucleotide bases specifically hybridizable with a selected sequence of RNA or DNA coding for said protein, and having at least one modified 2'-deoxyfuranosyl moiety. Modifications encompassed by the present invention include hydrogen, hydroxyl, halo, azido, amino, substituted amino, cyano, halomethyl, isocyanato, alkoxyl, thioalkoxyl, haloalkoxyl, alkyl sulfide, alkyl sulfonate, nitrate, nitrite, ammonium, allyloxy, or alkeneoxy. Oligonucleotides of the present invention may be further modified such that at least some of the sugar linking groups of said oligonucleotide are modified to comprise a phosphorothioate, methyl phosphonate, or phosphate alkylate. Applicants found that such sugar-modified oligonucleotides hybridized to the targeted RNA more strongly than did the unmodified type. (Page 17, lines 6-7). In addition, Applicants found melting temperatures increased with 2'-substituted adenosine diphosphates. (Page 16, lines 15-16).

Obviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination. *In re Fine*, 837 F.2d 1071 (Fed. Cir. 1988). Rather than suggesting a combination leading to the present invention, Marcus-Sekura et al. and Ikehara et al. teach away from the

present invention. Contrary to the Examiner's suggestion that the teachings of Marcus-Sekura et al. would motivate one to combine with Ikehara et al., this reference does just the opposite. By providing a solution to the problem of nuclease susceptibility, Marcus-Sekura removes the incentive for one skilled in the art to solve (or re-solve) the problem by the addition of 2' substituted furanosyl groups.

Even were one skilled in the art motivated to seek an additional source of nuclease resistance, it is unlikely that one would be motivated to incorporate substituted nucleotides as described in Ikehara et al. because the reference teaches that while exhibiting at least limited nuclease resistance, 2'-modified polynucleotides lack thermal stability. Thermal stability which is indicative of stable duplex formation is significant to antisense principles because the antisense mechanism is based upon stable duplex formation. Even a constituent which confers nuclease resistant will not be usefully incorporated within an antisense oligonucleotide if it interferes with the ability of the oligonucleotide to form duplexes with fidelity. Therefore, it would not be obvious in light of the teachings of Ikehara et al., to incorporate 2'-modified nucleotides into antisense compounds. Accordingly, Applicants respectfully request withdrawal of this § 103 rejection.

In light of the foregoing arguments, Applicants believe the claims, as amended, are in condition for allowance and an early and favorable action is earnestly solicited.

Respectfully submitted,

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